Mitochondrial Transplantation as a Potential Therapeutic for Osteoarthritis

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INTRODUCTION: Osteoarthritis (OA) is a prevalent degenerative joint disease characterized by progressive cartilage loss, joint pain, and functional impairment. Current treatments for OA primarily focus on symptomatic relief and fail to address the underlying pathophysiological mechanisms. Emerging evidence suggests that mitochondrial dysfunction plays a crucial role in the development and progression of OA, contributing to oxidative stress, inflammation, and impaired cellular metabolism within articular tissues. Mitochondrial transfer, a novel therapeutic strategy, offers a promising approach to ameliorate OA-associated mitochondrial dysfunction and restore cellular homeostasis. Our objective is to transfer exogenous mitochondria from young donors into aged cells to test the potential of this new approach in preventing premature cellular senescence, reducing inflammatory-related changes and promoting tissue regeneration of these recipient cells. Our hypothesis is that delivery of healthy mitochondria from young patient stem cells will confer improved metabolic performance of chondrocyte extracellular matrix synthesis.

METHODS:

Mitochondria were isolated from cultured human synovial-fluid derived stem cells obtained from a 15-year-old patient through differential centrifugation using a commercial kit (Thermo Scientific™, Waltham, MA). Isolation of the mitochondrial fraction was performed by adding a series of buffers provided in the kit, following the manufacturer protocol. Mitochondria were obtained from 2x107 cells with treatment via a series of kit reagents. The suspension was then centrifuged at 700 g for 10 minutes at 4°C, and the supernatant was centrifuged at 12,000g for 15 minutes. The mitochondria fraction was then resuspended in pH 7.4 respiration buffer containing 70 mM sucrose (ThermoFisher, L-12686), 220 mM mannitol (Sigma, M9546), 5 mM KH2PO4 (Sigma-Aldrich, P5655), 5 mM MgCl2 (Sigma, M0250), 1 mM EGTA (Sigma, E4378), 2 mM HEPES (Corning, 25-060-CI) with pH 7.4 using potassium hydroxide (KOH) to maintain mitochondrial integrity for downstream analysis. Initial work focused on labeling the mitochondria for tracking in vitro (Fig 1.). We then explored the ability of stem cells to uptake the mitochondria when cocultured (Fig. 2). Cells from a 60-year-old patient were then cocultured and analyzed to confirm mitochondrial transfer, and qRT-PCR was performed to determine collagen type II gene expression in a dose-dependent fashion. Subsequent experiments evaluated how IL-1b could potentially impact mitochondrial transfer and modeled an OA inflammatory milieu.

RESULTS: Young mitochondria were successfully isolated and labeled for tracking, as demonstrated by confocal microscopy (Fig. 2). When cocultured with chondrocytes from a 60-year-old patient, collagen type II and SIRT1, a cell survival marker and inflammation inhibitor, were upregulated in a dose-dependent fashion. Under inflammatory conditions with IL-1β, collagen II synthesis was maintained, albeit SIRT1 was moderately downregulated (Fig. 3).

DISCUSSION AND CONCLUSION: Overall, mitochondrial transfer holds significant promise as a novel therapeutic modality for OA by targeting the underlying mitochondrial dysfunction, thereby offering potential disease-modifying effects and improved outcomes for patients suffering from this debilitating condition. This represents the first proof of concept for a promising approach to cartilage repair as well as OA. Further work will examine a full complement of gene targets and transition to a preclinical model of OA.